Gene Expression in Microgravity

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Gene Expression in Space

- Osteoblasts (Dr. Millie Hughes-Fulford)
  - Decrease in c-fos,c-jun, and cox-2
  - Possible consequence of changes in cytoskeleton but not matrix protein
- Brain (Pompeiano et al.)
  - Immediate early genes (IEG)
  - Rat model (cerebellum, hypothalamus, spine
  - No results available

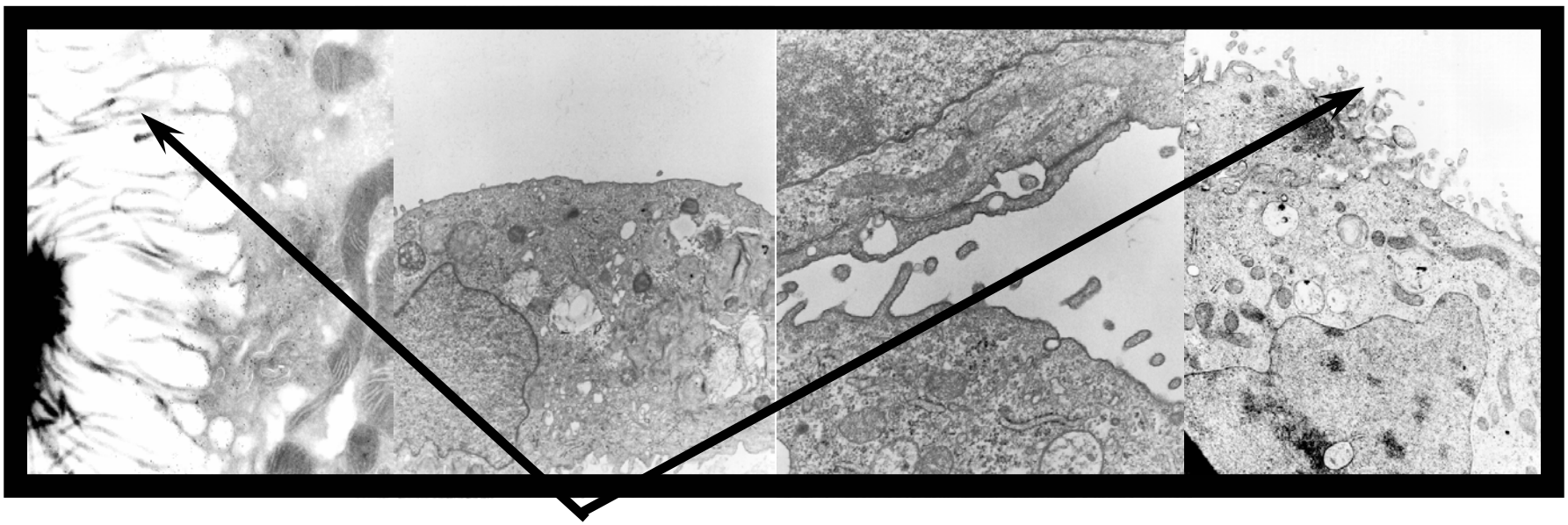
Gene Expression in Space

- Zebrafish (Gillette-Ferguson et al.)
  - Transgenic model with *gfp* gene under the control of the β-actin promoter
  - Microgravity effects show a 9% increase in *gfp* expression in the heart

Renal Proximal Tubular Cells

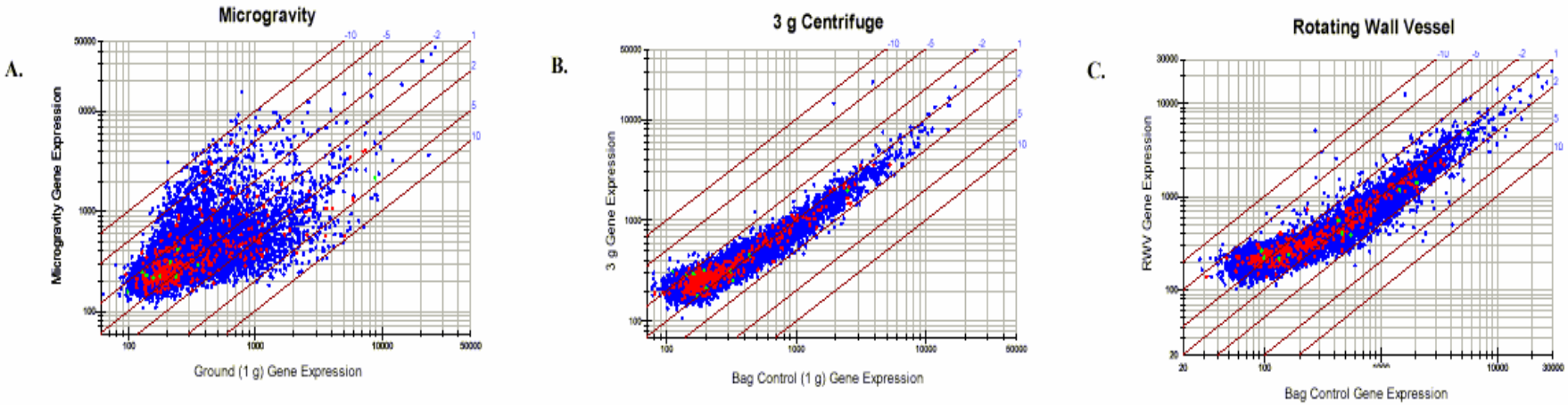
Dr. Timothy Hammond, Tulane Univ. Med.Ctr.

(An in vitro test for renal toxicity)



Microvilli with receptors for aminoglycosides
Synthesis of Vitamin D precursors

Gene Expression in Microgravity



- The response of human cells to microgravity is fundamental to the adaptation of terrestrial life to low gravity environments and is reflected in the genes tat are activated in space.
- As we observe the changes in cells in microgravity we open new opportunities into the fundamental processes in cells.
- Some of the arenas where the scientific community uses these findings is in the engineering of tissues for transplantation , modeling of disease, and the propagation of infectious organisms
- In May of 1999 Dr. Tim Hammond of Tulane University published his findings from microgravity and bioreactor experiments on the genes that are turned on and off when cells are transitioned to space (Nature Medicine 5/99)

Space versus analog

- Decreased compared to Analog
  - Adhesion molecule
  - Apoptosis
  - Cytoskeletal proteins
  - Differentiation
  - Intracellular signaling proteins
  - Receptors
  - Transcription factors

Space versus analog

- Increased compared to Analog
  - Electron transport
  - Stress
- Mixed
  - Nucleic acid synthesis and modification
  - Heat shock

Table 1. *Genes whose expression changed >3.0 in space with comparative results in the rotating wall vessel*

Gene Groups	IMAGE No.	Space	RWV
Adhesion			
Human laminin β2-chain (LAMB2)	359741	−3	2.1
Human platelet/endothelial cell adhesion	489123	−3.5	2
Human mRNA for integrin α6-subunit	159512	−4	2.6
Human carcino-embryonic antigen mRNA	510405	−6.4	2.3
Apoptosis			
Human tumor necrosis factor receptor 2	470493	−11.1	−1.4
Human cysteine protease Mch2 isoform	323500	−6.6	1.2
Human B94 protein mRNA; complete	487045	−5.8	−1.2
Human mRNA for mutated p53 transformation	236338	3	−1.3
Cytoskeletal proteins			
Human skeletal muscle α-tropomyosin	488479	−3.8	1.3
Human non-lens βγ-crystallin like	297589	−6	1.2
Human mRNA for plasma gelsolin	359675	8.2	1
Human mRNA for kinesin-related protein	327575	3.9	−1.7
Human mRNA for integrin α6-subunit	159512	−4	1.2
Human mRNA for cytokeratin	509980	−7.1	1.3
Human mRNA for coronin	487988	8.6	1.2
Human mRNA for β-centractin	381596	5.1	1.2
Human laminin B2 (LAMB2) mRNA; partial	485332	−3.5	1.4
Human keratin type II (58 kDa) mRNA; complete	345925	−5	−1
Human I-plastin mRNA; complete	381819	−3.1	1.2
Human dystrophin gene	294950	−3	−1.4
Human cytokeratin 18 mRNA; 3′ end	114872	−4.6	1
Human cardiac myosin heavy chain mRNA; 3	300069	3.5	1
Human β-tubulin pseudogene; complete	296731	−7.5	−1.1
Human α-spectrin gene; exon 52	427750	4.9	−1.1
Human α-cardiac actin gene; exon 6	485743	4.6	1.5
Human actin-related protein Arp3 (ARP3)	359910	5	1.6
Human (clone PWHLC2-24) myosin light chain	417479	−3.9	1
Differentiation			
vav; VAV	199381	−3.2	0
Rat developmentally regulated protein mRNA	360210	−3.6	1.3
Rat clone C101 intestinal epithelium protein	363123	−3.1	2.1
Human zinc finger/leucine zipper protein	113307	3	−1.1
Human Wilms' tumor (WT33) protein mRNA	470470	9.1	1.1
Human ubiquitin protease (Unph) proto-oncogene	486665	2.9	−1.2
Human triiodothyronine receptor (THRA1)	364080	−6.7	1.4
Human shorter form basic fibroblast growth factor	21955	−6.9	−1.3
Human set gene; complete	510260	−3.7	1
Human novel growth factor receptor mRNA	180447	−4.2	−1
Human nerve growth factor-β (β-NGF)	72869	−4.4	−1.4
Human mRNA for p0071 protein	52476	3.3	−1
Human mRNA for myoblast cell surface antigen	363658	4.6	1.1
Human membrane-associated protein (HEM-1)	322627	6	−1.1
Human JNK2 α1 protein kinase (JNK2A1)	322029	3.4	−1.5
Human IEF 9306 mRNA	429361	3.2	−1.1
Human homolog of *Drosophila* enhancer	469370	−3	−1.4
Human GM-CSF receptor mRNA; complete	140352	−7.7	−1.3
Human glycogen synthase kinase 3 mRNA	22047	4.8	1.2
Human dek mRNA	486647	−3	−1.7
Human CD34 mRNA; complete	213635	3	1
Human calcineurin B mRNA; complete	489081	3.2	1.1
Human c-syn proto-oncogene; complete	323555	5.1	−1.2
Human BTG1 mRNA	291035	4.2	−1
FMS; growth factor receptor; CSF-1 receptor	204653	6.7	1.1
C33 antigen; type III integral membrane protein	488596	−3	−1
Electron transport			
Thioredoxin	415014	3.4	1
NADH:ubiquinone oxidoreductase (428 AA)	509804	7	1.4
Human mRNA for electron transfer flavoprotein	308745	3	1.2
Human gene for very-long-chain acyl-CoA	428227	3.7	1.1
Human electron transfer flavoprotein alp	488221	3	1.2
Human cytochrome b561 gene; exon 6	376146	−3.6	−2
Human cytochrome b5 mRNA; complete	415153	5.2	1.2
ATR1; NADPH-ferrihemoprotein reductase	309770	−3	−1
Adhesion			
Intracellular signaling proteins			
Human tyrosine kinase-type receptor	365147	−5	−1
Human shorter form basic fibroblast growth factor	21955	−6.9	−1.3

Table 1.—*Continued*

Gene Groups	IMAGE No.	Space	RWV
Human set gene; complete	510260	−3.7	1
Human phosphatidylinositol 4,5-bisphosphatase	29804	4	−1.6
Human mRNA for rab 13	366489	−3.7	1.2
Human mRNA for putative serine/threonine	472138	6.2	1.4
Human mRNA for KIAA0118 gene; partial	151066	−3.5	−1.1
Human GTP binding protein (ARL3) mRNA	360902	−3	1.1
Human glycogen synthase kinase 3 mRNA	22047	4.8	1.2
Human cyclin E (CCNE) gene; exon B	357807	−3.9	1.6
Human calcineurin B mRNA; complete	489081	3.2	1.1
Nucleic acid synthesis and modification			
Human RNA polymerase II subunit hsRPB4	302293	−3.5	1.5
Human mRNA for myoblast cell surface antigen	363658	4.6	1.1
Receptors			
OB-R gene related protein (OB-RGRP)	323210	−3.2	1.1
Human vitamin D receptor mRNA; complete	365566	−7.4	1.1
Human tyrosine kinase-type receptor	365147	−7.4	1.1
Human tumor necrosis factor receptor-2	470493	−5	−1
Human triiodothyronine receptor (THRA1)	364080	−11.1	−1.4
Human transforming growth factor-β	209655	−6.7	1.4
Human shorter form basic fibroblast growth factor	21955	−6.6	−1
Human putative G-protein-coupled receptor	345973	−6.9	−1.3
Human OB-RGRP gene	265571	−4.7	−1.4
Human novel growth factor receptor mRNA	180447	−5.6	1.2
Human mRNA for T-cell receptor β-chain	302157	−4.2	−1
Human mRNA for interferon α/β receptor	123950	−3.6	−1.1
Human mRNA for growth hormone receptor	295389	3.6	−1.1
Human lectin-like type II integral membrane protein	415086	−4.7	1
Human GM-CSF receptor mRNA; complete	140352	−3.2	−1.6
Human endothelial cell protein C/APC receptor	376268	−7.7	−1.3
Human DNA for human P2XM; complete	60566	−6.3	1.6
Human 180-kDa transmembrane PLA_2 receptor	320355	−3.7	1.4
FMS; growth factor receptor; CSF-1 receptor	204653	−3.4	1.3
Stress			
ESTs	509962	6.7	1.1
Superoxide dismutase	417829	4.1	1.2
Transcription factors			
Pzf; zinc finger protein	297856	−6.2	−1.1
Human ZNF81 gene	229467	−6.1	1
Human zinc finger/leucine zipper protein	113307	3	−1.1
Human zinc finger protein ZNF136	343096	−4.1	1
Human zinc finger protein mRNA; complete	61531	−3.4	1.1
Human zinc finger protein (LD5-1) mRNA	417295	−3	1
Human Wilms' tumor (WT33) protein mRNA	470470	9.1	1.1
Human vitamin D receptor mRNA; complete	365566	−7.4	1.1
Human triiodothyronine receptor (THRA1)	364080	−6.7	1.4
Human transcription factor TFIIA small subunit	485192	3	−1.8
Human transcription factor (ITF-2) mRNA	380738	5.6	0
Human TFII-I protein (TFII-I) mRNA; complete	376973	−3.6	−1.4
Human sequence-specific DNA-binding protein	293032	−7.1	1
Human mRNA for RNA polymerase II association	323480	−6.5	1
Human mRNA for mutated p53 transformation	236338	3	−1.3
Human mRNA for KIAA0262 gene	485008	4.5	1
Human interferon regulatory factor 3	203017	3	1.1
Human IEF 9306 mRNA	429361	3.2	−1.1
Human EWS-E1A-F chimeric protein mRNA	363805	3.8	1.5
Human dek mRNA	486647	−3	−1.7
Human aryl hydrocarbon receptor nuclear	324799	6.3	1.1
Drug metabolism			
Human P-glycoprotein (MDR1) gene; exon 1	39920	−3.5	1.1
Human glutathione *S*-transferase (GSTM5)	377731	3.4	−1.5
Human glutathione *S*-transferase (GST)	365434	−4.3	1.6
Heat shock			
Human tra1 mRNA	242829	−5.5	−1.2
Human heart mRNA for heat shock protein	510320	3.1	1.1

Gene expression changes in space. Genes whose expression changes more than 3-fold in space are listed according to gene groups. For comparison, changes during rotating wall vessel (RWV) culture in the same genes are also listed. The values shown represent balanced differential gene expression. Gene expression is reported as the difference between the cyanine-3 fluorescent signal in the control cDNA sample and cyanine-5 signal on the experimental cDNA sample. The experimental cDNA sample is corrected for differences in total signal strength based on internal controls on the gene chip. See Ref. 8a for description of IMAGE nos. (see also **http://image.llnl.gov/**).

Mechanical culture conditions effect renal cell gene expression

ISS Utilization-2001

Tulane Environmental

Astrobiology Lab

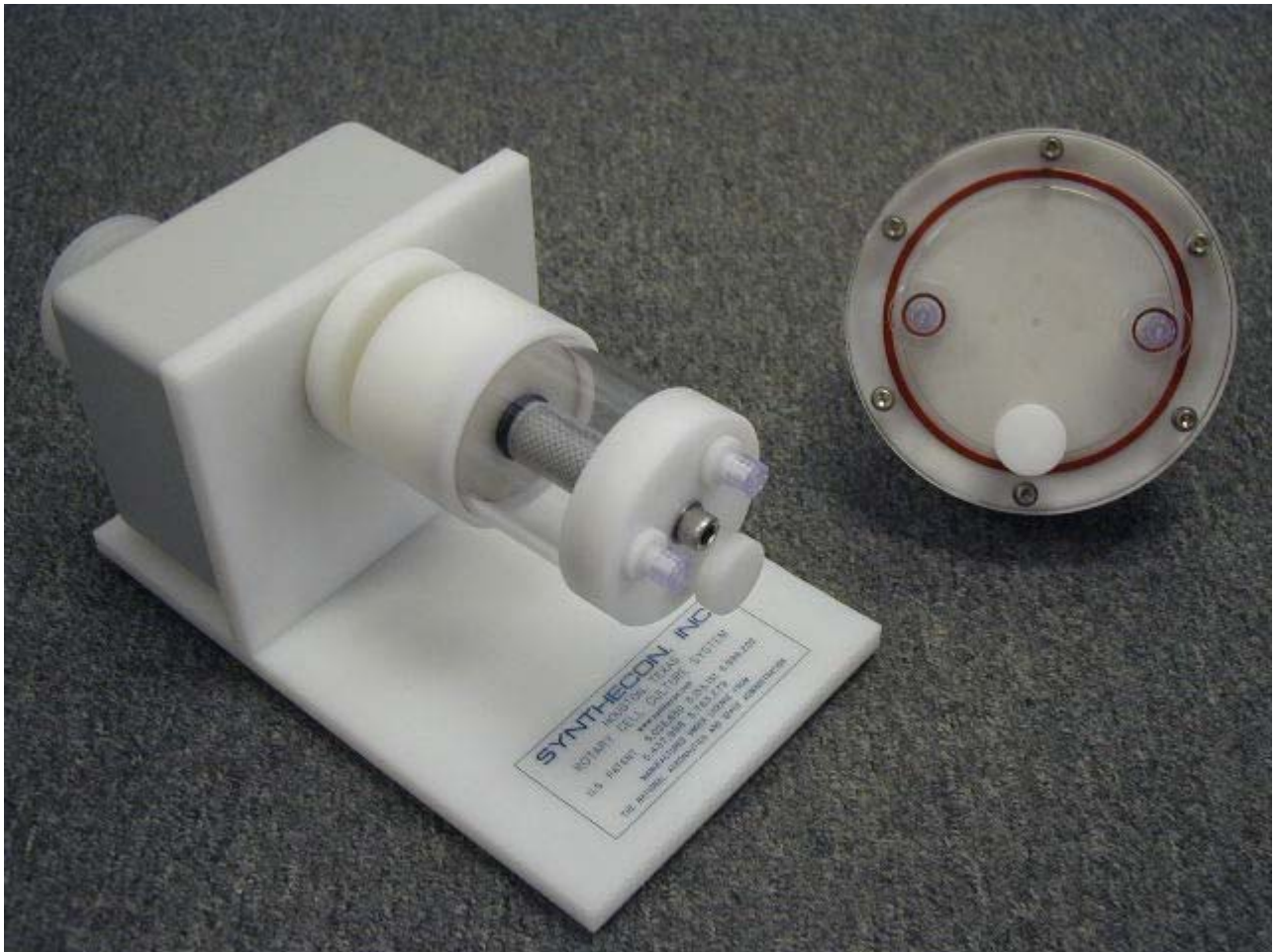
October 16, 2001

Flight experiment

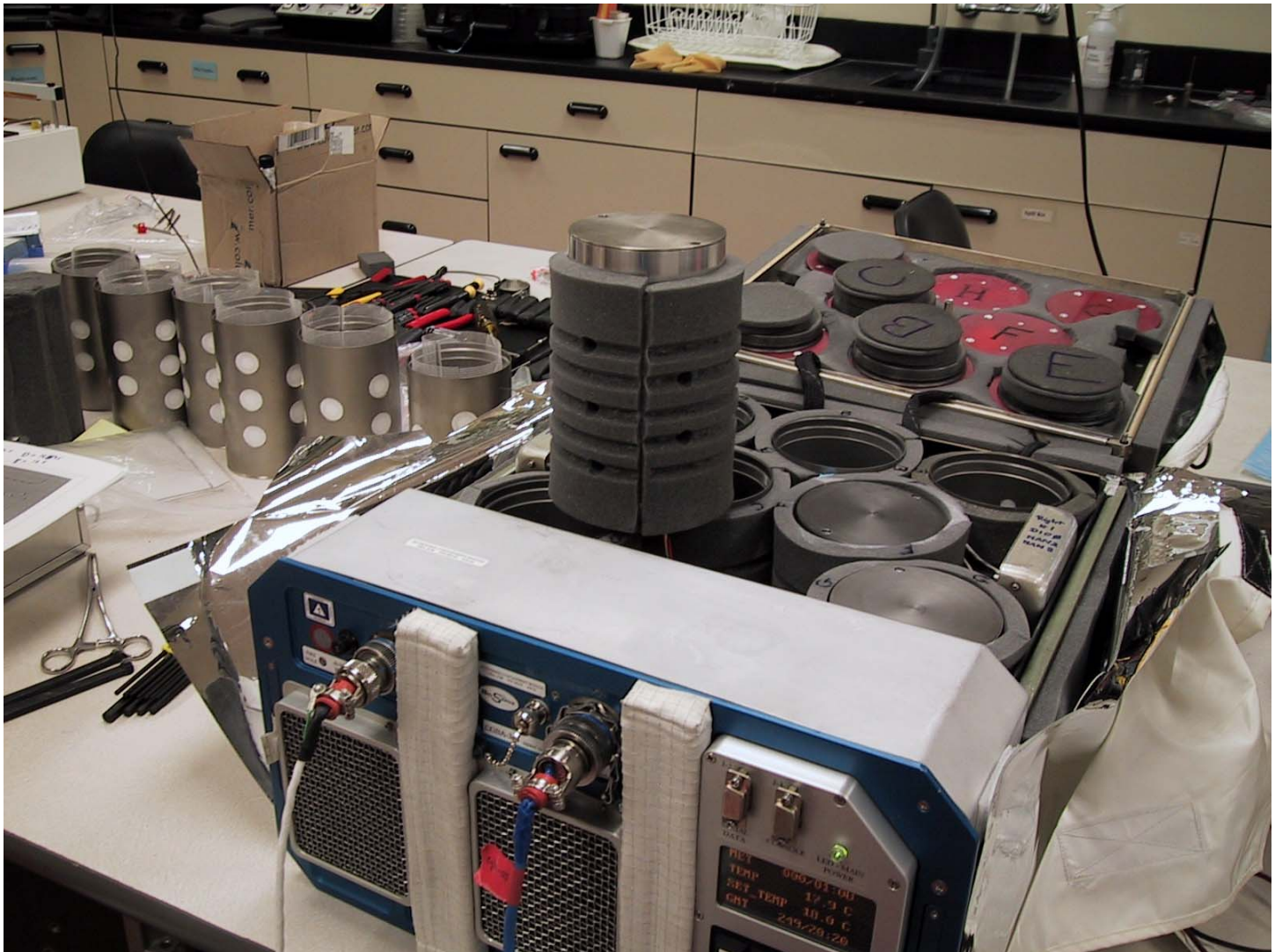
- To characterize renal cell gene expression changes during space flight
- to dissect gene expression changes due to vibration, gravity of launch and compare to rotating wal vessel
- to predict conditions to make bioproducts


HRCE STS-106
36

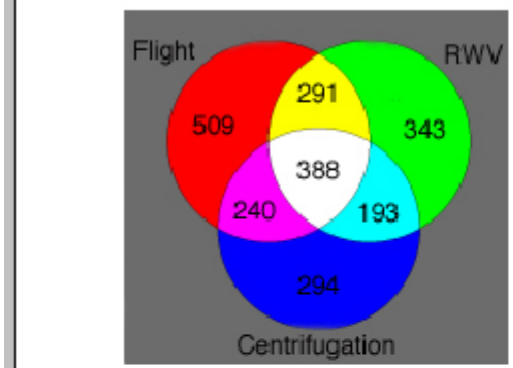
Rotating wall vessel

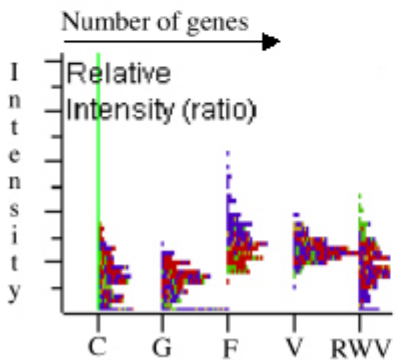


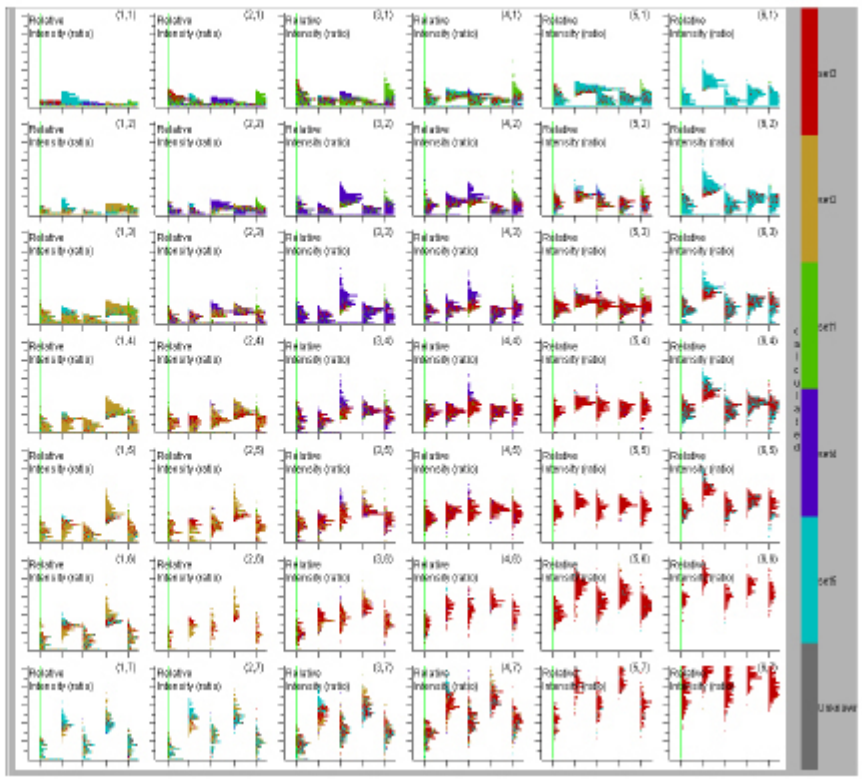
BIOSERVE

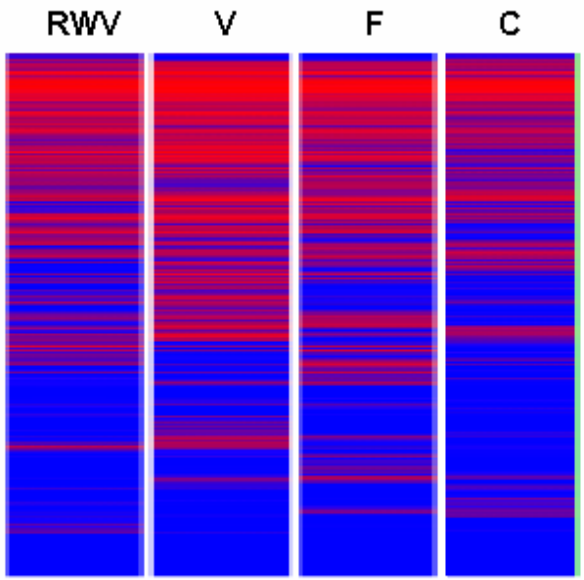


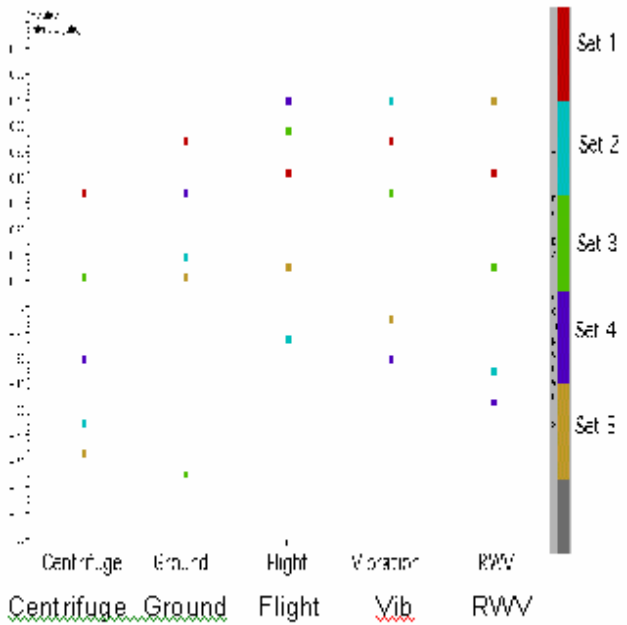
Gene array clusters

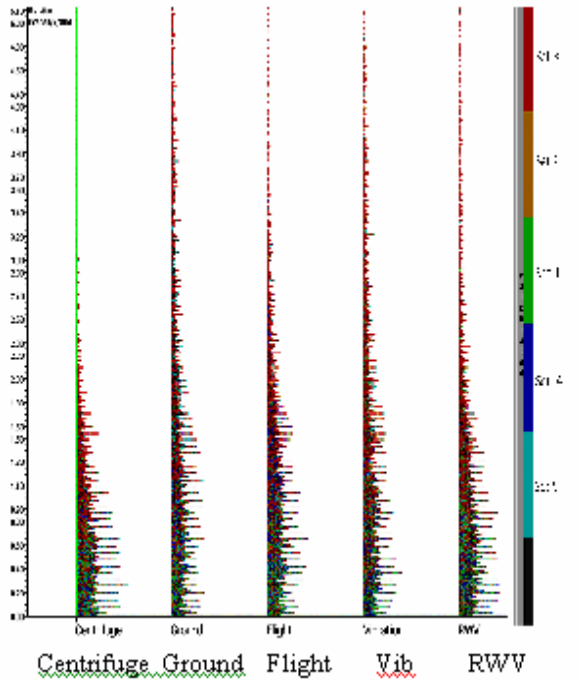


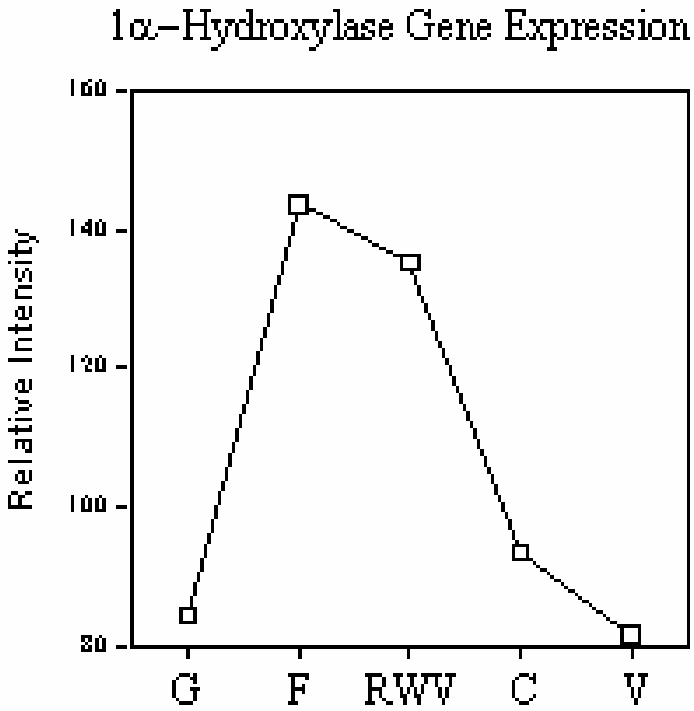


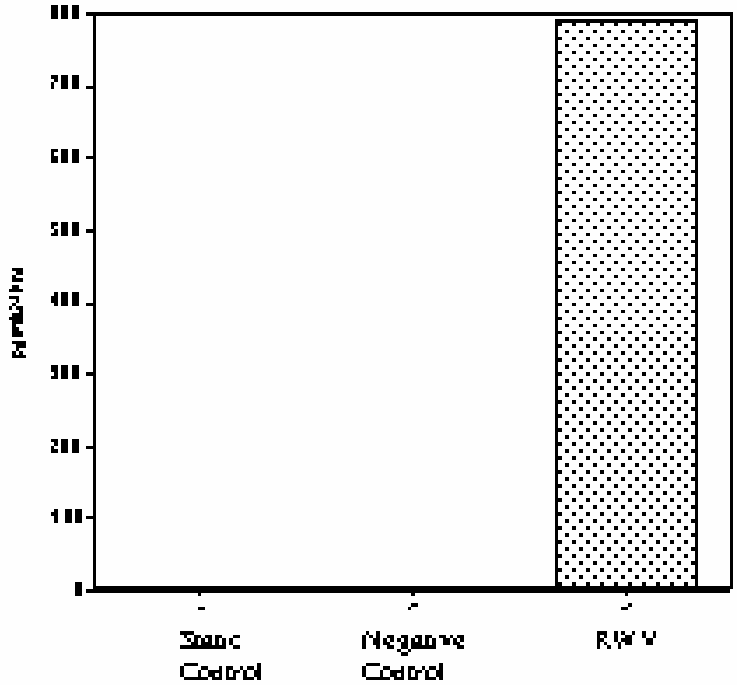


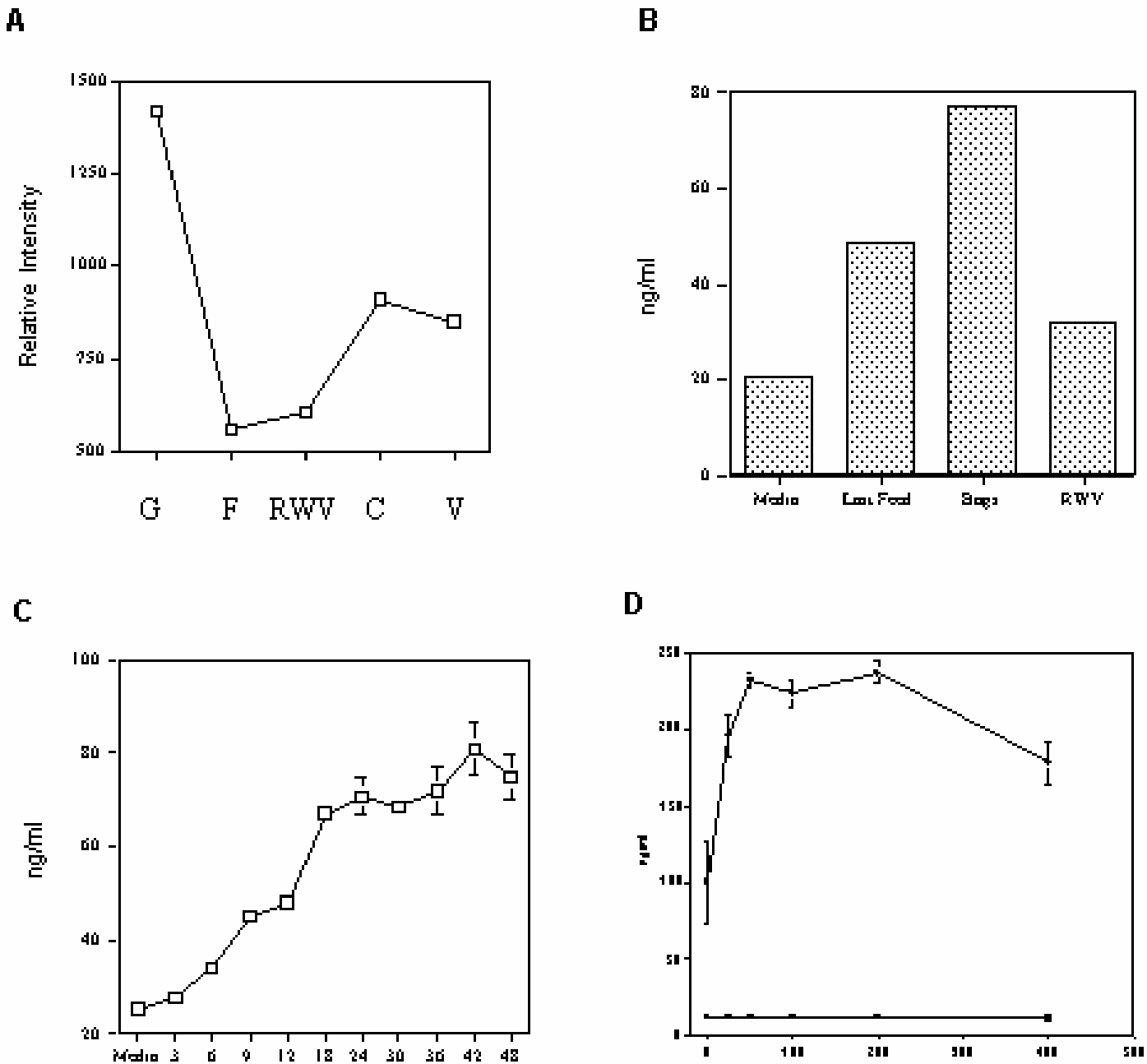

RWV
V
F
C

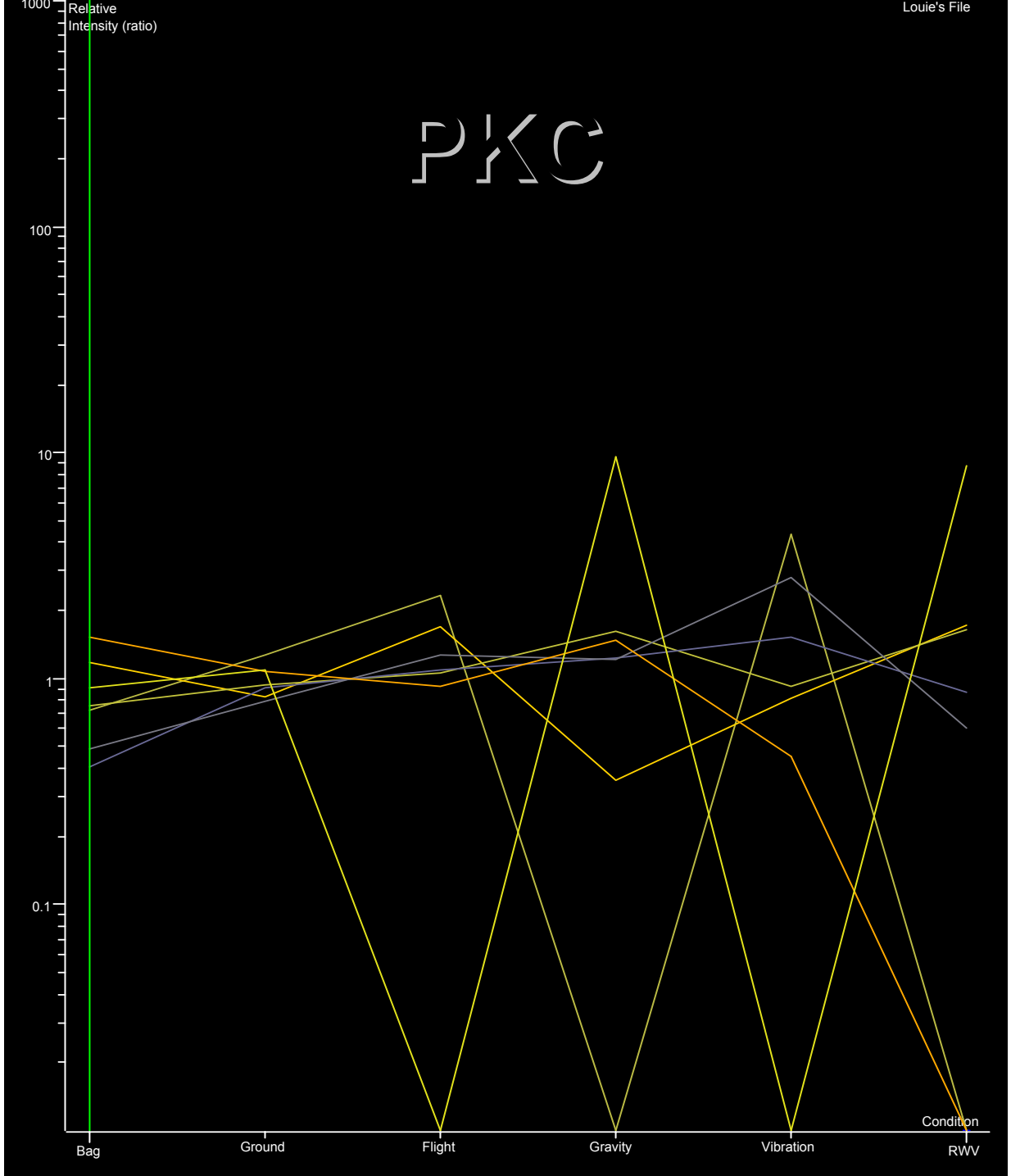

Set 1
Set 2
Set 3
Set 4
Set 5
Centrifuge
Ground
Flight
Vib
RWV


Centrifuge
Ground
Flight
Vib
RWV


1α-Hydroxylase Gene Expression
Relative Intensity
160
140
120
100
80
G
F
RWV
C
V


Static Control
Negative Control
RWV


A
Relative Intensity
1500
1250
1000
750
500
G
F
RWV
C
V
B
ng/ml
80
60
40
20
0
Media
Lact Feed
Stags
RWV
C
ng/ml
100
80
60
40
20
Media
3
6
9
12
18
24
30
36
42
48
D
250
200
150
100
50
0
0
100
200
300
400
500


Relative
Intensity (ratio)
Louie's File
PKC
1000
100
10
1
0.1
Condition
Bag
Ground
Flight
Gravity
Vibration
RWV

Bioproduct production conditions

- Vitamin D : rotating wall vessel
- Interferon in flight
- Other - in intellectual property review

Conclusions

- Microgravity is a profound physical perturbation to cells
- The response suite in space is vast
- The actual contribution of microgravity to the response suite is undoubted smaller than the observations in space cell culture
- It is anticipated that mammalian cells will respond differently than plants and bacteria